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In vitro Response of Glycerol Metabolism to Insulin and Adrenaline in Adipose Tissue from Fed and Fasted Rats during Pregnancy

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Abstract. Pieces of lumbar adipose tissue from 19-day pregnant rats and their virgin controls were incubated in the presence of albumin, glucose, U-^{14}C-glycerol, and either bovine insulin (200 μU/ml) or adrenaline (2.6 μM). The rate of glycerol release in the medium was augmented in both fed and 48-hour fasted pregnant rats. Insulin reduced this parameter in tissues from pregnant rats but not from controls. Adrenaline enhanced it in all groups, especially in tissues from fed pregnant rats. The rates of CO_2, fatty acids and glyceride glycerol formation from glycerol were higher and the effect of insulin was greater in pregnant than in control rats when fed. Fasting produced a decrease in all these parameters, the effect being greater in pregnant than in control rats. The augmented sensitivity of adipose tissue from the mother allows for a rapid switch from the anabolic to the catabolic state according to the necessities of the fetus.

Introduction

The enhanced lipolysis in adipose tissue during late pregnancy (20, 22) is counteracted by an intense lipogenetic activity (22) allowing the net deposition of fat stores in the mother (1, 18, 22). We have recently shown that beside the use of glucose as a lipogenetic substrate (22, 24), the utilization of glycerol by the adipose tissue from pregnant rats is augmented (4). Glycerol metabolism in adipose tissue is intensely affected by insulin and adrenaline (3, 21, 32) and it is well known that the sensitivity of different parameters to insulin is altered during pregnancy (2, 23, 25). In the present study, the *in vitro* response of adipose tissue glycerol metabolism to both insulin and adrenaline was investigated in pregnant rats and their virgin controls. As starvation produces intense changes in the lipidic metabolism during pregnancy (6, 22, 28) and it is known to affect the glycerol metabolic activity in adipose tissue (4, 16), the study was performed in both fed and 48-hour starved animals.

Materials and Methods

19-day pregnant Wistar rats and age matched, female virgin controls were maintained in a temperature (22 ± 2 °C) and light cycle (12 h on-off) controlled environment. The date of pregnancy was determined by the presence of spermatozoids in the vagina. Following *ad libitum* feeding or a 48-hour starvation period with free access to drinking tap water, all animals were killed by decapitation. Lumbar adipose tissue was dissected and the fat pad pieces were incubated *in vitro* in Krebs-Ringer bicarbonate medium pH 7.4 (31) containing bovine albumin (fraction V from Sigma) purified by the method of *Chen* (5), 10 μM U-^{14}C-glycerol (0.5 μCi/ml) and 5 mM glucose. The medium was supplemented with glucagon-free bovine insulin (200 μU/ml, generously supplied by Novo Industria A/S) or adrenaline (2.6 μM)

Table I. *In vitro* glycerol release and U-^{14}C-glycerol uptake (expressed as percentage of initial radioactivity in the medium) by pieces of lumbar fat pads from fed and 48-hour fasted 19-day pregnant rats and their virgin controls, incubated for 180 min

	Glycerol in media at 180 min μmol/mg protein	Uptake of U-^{14}C-glycerol at 180 min %/mg protein
Fed virgin (controls)	0.206 ± 0.017	15.40 ± 2.15
Fed pregnant	0.323 ± 0.021	17.75 ± 3.21
p	<0.01	NS
Fasted virgin (controls)	0.203 ± 0.015	3.54 ± 0.32***
Fasted pregnant	0.410 ± 0.025*	2.05 ± 0.41***
p	<0.001	<0.05

Data represent the mean ± SEM of 6 rats per group. p = Statistical comparison between pregnant and virgin animals; while comparison between fed and fasted groups: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table II. Rates of *in vitro* lipolysis (glycerol released in nmol/min/mg protein) by pieces of lumbar fat pads from fed and 48-hour fasted 19-day pregnant rats and their virgin controls, incubated in the presence of either insulin (200 μU/ml) or adrenaline (2.6 μM)

	Basal	p'	+ Insulin	p'
Fed virgin (controls)	1.44 ± 0.11	NS	1.21 ± 0.12	<0.001
Fed pregnant	2.01 ± 0.17	<0.05	1.70 ± 0.14	<0.001
p	<0.01		<0.01	
Fasted virgin (controls)	1.34 ± 0.14	NS	1.27 ± 0.10	<0.001
Fasted pregnant	2.68 ± 0.19*	<0.001	1.52 ± 0.11	<0.001
p	<0.001		NS	

Data represent the mean ± SEM of 30 values per group. p = Statistical comparison between pregnant and virgin animals; p' = comparison of tissues incubated in the presence of each hormone and their basals; while the comparison between fed and fasted groups is: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

where indicated. The incubations were carried out as previously described (14, 15) and were stopped by injecting $HClO_4$ into the vial. After collecting the evolved $^{14}CO_2$ into hydroxide of hyamine (X10), the glycerol in the media was analyzed by an enzymatic procedure (12) and the lipids in the tissues were extracted and purified (10) and fractionated to determine radioactivity in the fatty acids and glyceride glycerol fractions (14, 15). Results were expressed per milligram of tissue protein which was determined by the method of *Lowry et al.* (26). The rates of glycerol release into the media (lipolysis) and its utilization by the tissues were calculated (4, 15) and statistical comparison of the groups was determined by means of a t test (9). Mathematical analysis of the data was performed with a Compucorp electronic calculator Model 445.

Results

The appearance of glycerol in the media and the uptake of U-^{14}C-glycerol by lumbar fat pad pieces from fed and 48-hour fasted 19-day pregnant rats and their virgin controls are shown in table I. It may be seen that the glycerol in the media was enhanced in both fed and fasted pregnant rats as compared with controls while the uptake of U-^{14}C-glycerol did not differ between the tissues of both groups when fed. Starvation produced an intense and significant reduction in the amount of labelled glycerol taken up by the tissues in both groups but the effect was greater and was statistically significant in pregnant versus control rats.

+ Adrenaline	p'	+ Insulin and adrenaline
5.03 + 0.45	<0.001	7.15 ± 0.40
7.51 + 0.31	<0.001	7.52 ± 0.32
<0.001		NS
3.40 + 0.32**	<0.001	3.46 ± 0.33***
3.32 + 0.21***	<0.001	3.39 + 0.33***
NS		NS

The varied dilution of labelled glycerol in the media during incubation time among the groups, due to differences in glycerol release from the tissues into the media, necessitated rate corrections of both lipolysis and glycerol utilization by the tissues. These calculations were performed as previously described (14, 15). The rates of lipolysis remained constant throughout the incubation time in all groups and their mean values are shown in table II. When incubated in basal conditions, tissues from pregnant rats showed more active lipolysis than did their controls, independent of the animals' dietary condition. With insulin in the media, the rate of lipolysis was significantly reduced in the tissues of pregnant rats whether fed or starved, while it was unchanged in controls. Adrenaline produced a significant increase in the rate of lipolysis in all groups with the greatest effect in tissues from fed pregnant rats. The adrenaline effect is less in the tissues from starved animals than fed ones, and there was no difference between starved pregnant or control rats. In the presence of both insulin and adrenaline, there was a further increase in the rate of lipolysis in tissues from fed controls as compared to those with adrenaline alone. This effect was not seen in tissues from pregnant rats.

The rate of glycerol metabolization by the tissues is a linear function of the concentration of glycerol in the media (4). In table III, the values correspond to the 50-μM concentration of glycerol in the media for all groups, to avoid

Table III. Rates of *in vitro* glycerol utilization (nmol/min/mg protein) for the formation of CO_2, fatty acids and glyceride glycerol, at a concentration of 50 μM, by pieces of lumbar fat pads from fed and 48-hour fasted 19-day pregnant rats and their virgin controls, incubated in the presence of either insulin (200 μU/ml) or adrenaline (2.6 μM)

	Basal	p′	+ Insulin	p′
CO_2				
Fed virgin (controls)	0.642 ± 0.007	< 0.05	0.996 ± 0.011	< 0.001
Fed pregnant	1.09 ± 0.12	NS	1.47 ± 0.16	< 0.001
p	< 0.01		< 0.01	
Fasted virgin (controls)	0.099 ± 0.008***	< 0.01	0.175 ± 0.031***	< 0.001
Fasted pregnant	0.023 ± 0.002***	< 0.001	0.136 ± 0.022***	< 0.001
p	< 0.001		NS	
Fatty acids				
Fed virgin (controls)	0.505 ± 0.051	< 0.001	1.06 ± 0.11	< 0.001
Fed pregnant	0.852 ± 0.085	< 0.001	1.73 ± 0.23	< 0.001
p	< 0.01		< 0.05	
Fasted virgin (controls)	0.031 ± 0.002***	NS	0.041 ± 0.006***	< 0.001
Fasted pregnant	0.005 ± 0.001***	< 0.001	0.039 ± 0.006***	NS
p	< 0.001		NS	
Glyceride glycerol				
Fed virgin (controls)	1.63 ± 0.17	< 0.01	1.08 ± 0.10	< 0.001
Fed pregnant	3.19 ± 0.38	< 0.001	2.04 ± 0.26	< 0.001
p	< 0.001		< 0.01	
Fasted virgin (controls)	0.407 ± 0.038***	NS	0.329 ± 0.054***	< 0.001
Fasted pregnant	0.243 ± 0.018***	< 0.05	0.369 ± 0.058***	< 0.001
p	< 0.001		NS	

Data represents the mean ± SEM of 6 rats/group. p = Statistical comparison between pregnant and virgin animals; p′ = comparison of tissues incubated in the presence of each hormone and their basals; while the comparison between fed and fasted groups is *** $p < 0.001$.

presenting the differing effects of other concentrations of this substrate in the media. The rates of CO_2, fatty acids, and glyceride glycerol formation from glycerol were higher in the tissue from pregnant rats than from fed controls. Following 48 h of starvation, these parameters decreased in both groups, most significantly in pregnant rats, their values appearing lower than those of controls. Insulin enhanced the rate of CO_2 production from glycerol in both groups when fasted and that of fatty acids when fed. Insulin decreased the formation of glyceride glycerol in both groups when fed and enhanced it in pregnant rats when fasted. In the presence of adrenaline, there was a marked reduction in the rate of glycerol metabolization in all groups but all of the parameters studied remained higher in the tis-

+ Adrenaline	p'	+ Insulin and adrenaline
0.055 ± 0.006	< 0.001	0.102 ± 0.00
0.113 ± 0.013	< 0.001	0.132 ± 0.01
< 0.001		< 0.05
0.000 ± 0.000***	< 0.001	0.000 ± 0.000***
0.000 ± 0.000***	< 0.001	0.000 ± 0.000***
NS		NS
0.017 ± 0.002	< 0.001	0.035 ± 0.003
0.031 ± 0.003	< 0.001	0.055 ± 0.004
< 0.001		< 0.001
0.007 ± 0.001***	< 0.001	0.010 ± 0.001***
0.005 ± 0.001***	< 0.001	0.011 ± 0.001***
NS		NS
0.227 ± 0.031	< 0.001	0.281 ± 0.022
0.504 ± 0.005	< 0.001	0.441 ± 0.031
< 0.001		< 0.001
0.140 ± 0.013***	< 0.001	0.121 ± 0.012***
0.066 ± 0.005***	< 0.001	0.131 ± 0.021***
< 0.001		NS

sues from pregnant than from control rats when fed. In the starved situation, the glyceride glycerol formation was reduced in pregnant versus control rats in the presence of adrenaline. Insulin in the presence of adrenaline did not alter these relationships although it caused the disappearance of group differences in glyceride glycerol formation in the tissues from starved animals.

Discussion

In agreement with previous results (4), the present study demonstrates that adipose tissue from fed, pregnant rats not only shows an enhanced lipolytic activity when incubated *in vitro* but an augmented ability to metabolize glycerol. The effect is mainly observed in the synthesis of glyceride glycerol from this parameter which indicates that an increased esterification of fatty acids with α-glycerophosphate coming from free glycerol may contribute to the net deposition of fat in the mother. Although the *in vivo* implications of these results remain to be established, the high concentration of plasma glycerol in the mother (13) would contribute to this effect, as it is known that glycerol utilization by adipose tissue *in vitro* is a function of the availability of glycerol (7). The unchanged lipolytic activity and reduced glycerol utilization in the tissues from pregnant rats when fasted induces a maximal net breakdown of glycerides and may contribute to the well-known lipid mobilization in the pregnant rat when food is withheld (6, 22, 28).

In contrast to the hypoglycemic effect of insulin which is decreased in pregnancy (2, 22) in the present study, this hormone's antilipolytic action, enhancement of fatty acid synthesis from glycerol in both fed and fasted rats and reduction of glyceride glycerol in fed animals were greater in pregnant than in control tissues. The increased insulin effect on glycerol metabolism in *in vitro* adipose tissue from pregnant rats is in agreement with the enhanced effect of this hormone on other parameters from a similar preparation, including the adipose tissue gas exchange (25) and the lipogenesis from glucose (22). It would also support the hypothesis that the mother's augmented insulinemia (11, 17, 24) may actively contribute to her net deposi-

tion of fat stores. This situation in the pregnant rat may be similar to that of enhanced insulinemia and a reduced hypoglycemic insulin effect reported in obese, hyperglycemic mice (29, 33) in which the net deposition of fat stores is also augmented. In this preparation, enhanced glycerol utilization by adipose tissue has also been reported, due to increased glycerokinase activity (30). Thus increased lipemia in these two conditions, obesity and pregnancy, may contribute to the reduced sensitivity of insulin to decreased blood glucose levels but does not affect its sensitivity to adipose tissue metabolism.

The lipolytic effect of adrenaline is greater in the adipose tissue from fed pregnant than control rats during a similar inhibition of glycerol utilization. Increased sensitivity of the mother's adipose tissue to lipolytic and antilipolytic factors allow her a rapid switch from the anabolic to the catabolic state according to the needs of the fetus, as has been previously proposed for other metabolic parameters (11).

In the presence of insulin, the lipolytic effect of adrenaline in the fed control rats is enhanced probably because, in this condition, the intracellular accumulation of fatty acids during incubation is reduced due to increased glucose utilization for their esterification (21). The synergic effect of insulin and adrenaline on lipolysis is not observed in tissues from pregnant animals probably because they are already performing lipolysis at maximal capacity. The lipolytic effect of adrenaline is minimal in the tissues from starved animals and is not altered by addition of insulin. Although further studies are required for conclusive interpretation of these findings, the possibility exists that an intracellular accumulation of free fatty acids and/or a decreased availability of energy could be responsible for the reduced sensitivity of the adipose tissue from starved animals to the lipolytic stimulus. Indeed it has been proposed (19) that these two factors may intensely affect lipolytic activity and sensitivity of the tissue, and both are known to be altered in the starved situation (21, 27). The decreased availability of energy in the adipose tissue from starved animals also corresponds to the reduced utilization of glycerol by these tissues as we have recently shown that this parameter is highly sensitive to the availability of energy (8).

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